Controlled reperfusion using a simplified perfusion system preserves function after acute and persistent limb ischemia: A preliminary study

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Objective: **Reperfusion of the limb after acute and persistent ischemia is associated with high rates of morbidity and mortality despite complete revascularization. Although reperfusion is a prerequisite for maintaining limb function, it may in itself cause further injury. There is experimental evidence that modification of the initial reperfusion modalities can minimize this reperfusion injury. We hypothesized that controlled reperfusion using a simple blood bag perfusion system reduces reperfusion injury and facilitates the return of normal function.**

Methods: **Fifteen consecutive patients (mean age, 80.5 ± 5.0 years) with severe, acute lower-limb ischemia were allocated to two treatment arms in this prospective, controlled observational study. Group I (n = 8) underwent surgical embolectomy alone, and group II (n = 7) underwent surgical embolectomy plus controlled reperfusion using a simplified perfusion system. Indication for controlled reperfusion was made by the responsible surgeon. Controlled reperfusion consisted of a 30-minute infusion of a crystalloid reperfusion solution that was mixed with oxygenated blood (the blood:reperfusion solution ratio was 6:1) distal to the occlusion. Duration of ischemia, postoperative amputation rate, motor function of the ischemic limb, and pre- and postoperative serum creatine kinase levels were assessed.**

Results: **The duration of ischemia was 10.7 ± 1.1 hours in group I and 19 ± 5.2 hours in group II ($P < .05$). The site of the arterial occlusion was the iliac artery in nine patients and the common femoral artery in six patients. Full recovery was achieved in six of seven patients in group II and in only two of eight patients in group I ($P < .05$). There were three in-hospital deaths in group I, and two patients underwent major amputations. No in-hospital deaths or major amputations occurred in group II.**

Conclusion: **The results from this preliminary study strongly suggest the hypothesis that the results of conventional embolectomy for acute, severe lower-limb ischemia can be improved by controlled reperfusion. To prove our preliminary findings, a large randomized, prospective, controlled, multicenter trial, the Controlled Reperfusion of the Acutely Ischemic Limb trial (CRAIL-Trial) is currently being conducted to prove our preliminary findings. (J Vasc Surg 2005; 42:690-4.)**

Persistent and acute ischemia of the extremity, including neurologic dysfunction of the compromised leg, is associated with high morbidity and mortality. The most common reasons for acute limb ischemia are embolism of cardiac or arterial origin and in situ thrombosis of arteriosclerotic vessels.[1] Since the late 1960s, surgical revascularization with use of the Fogarty catheter primarily has been the therapeutic gold standard. It must be emphasized that the results of surgical therapy have not improved over the decades.[2] Even the introduction of new interventional treatment options such as intra-arterial thrombolysis did not reduce the high rates of mortality and amputation.[3-5]

Crucial in treating acute lower-limb ischemia is that restoration of arterial blood flow, essential for limb salvage, can further damage ischemic tissue in a phenomenon known as reperfusion injury.[6] Reperfusion injury and its systemic effects on remote organs can cause severe local and systemic complications such as renal and pulmonary failure.[7-9] There is experimental evidence that modifying the initial perfusion modalities, especially perfusion pressure and composition of the initial perfusate, can reduce reperfusion injury.[10]

The therapeutic principle named "controlled reperfusion" was first used to treat myocardial ischemia.[11] Reduction of the initial reperfusion pressure is aimed at reducing edema development, the modification of the initial reperfusate is aimed at counteracting the known biochemical changes that occur with ischemia-reperfusion, such as the break down of aerobic metabolism, metabolic acidosis, an increase in intracellular calcium, and the development of oxygen-derived free radicals with the onset of reperfusion.

In an animal model of acute lower-limb ischemia, local and systemic complications were reduced by the use of controlled reperfusion .[12] The concept of controlled reperfusion has been successfully used in clinical practice for treating patients with severe, prolonged lower-limb ischemia.[13-16]

All techniques described so far have required the use of a heart-lung machine or roller pumps. We have developed a new blood bag reperfusion system that allows the application of controlled reperfusion on acutely ischemic limbs with minimal technical effort.[17] As yet, no prospective study has been published to prove the beneficial effects of

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controlled limb reperfusion in patients with acute lower-limb ischemia. We hypothesized that controlled reperfusion using a simple blood bag perfusion system reduces reperfusion injury and thus facilitates the return of normal function.

METHODS

Study protocol. Fifteen consecutive patients presenting with acute and persistent severe lower-limb ischemia were included in the study. All patients showed ischemia-induced neurologic dysfunction (sensory, motor, or both) of the involved leg, thus meeting the criteria for class II or III according to the suggestions by the Society for Vascular Surgery.[18]

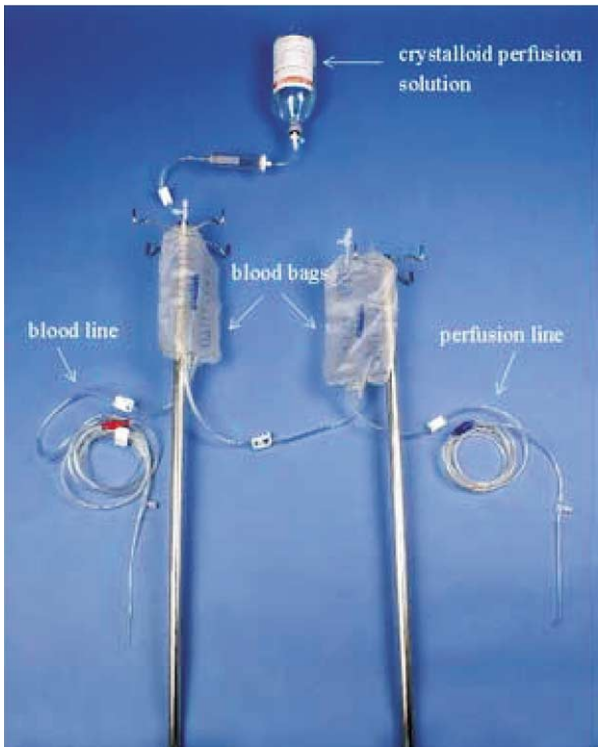
The decision for surgical revascularization alone (group I) or surgical revascularization plus controlled reperfusion (group II) was made by the responsible surgeon. Because this was a preliminary study, the decision for controlled reperfusion was based on the surgeon's opinion of which patients would benefit most from a modified therapeutic approach.

The study was conducted at a university and two academic teaching hospitals. All in-hospital deaths, major amputations, and neurologic function of the involved extremity at discharge from the hospital were assessed as major endpoints. Serum creatine kinase (CK) levels were evaluated preoperative and 24 hours postoperative. The study protocol was approved by the University of Freiburg Ethics Committee. All patients gave written informed consent.

Management of controlled reperfusion. The perfusion set for applying controlled limb reperfusion consisted of two blood bags (each capable of holding 1 liter), crystalloid solution, a blood line, and a reperfusion line (both made of .25-inch polyester tubes) (Fig). Controlled limb reperfusion was performed as follows: the common, superficial, and deep femoral arteries were exposed through a standard groin incision. After putting the arteries on vessel loops, a standard embolectomy or thrombectomy was performed using a Fogarty catheter with longitudinal or vertical incision of the vessel. Before restoration of blood flow, a 10-Charrier (CH) (1 CH is equivalent to .33 mm) cannula was inserted proximally into the iliac artery, another 10-CH cannula was inserted distally into the femoral artery through the same incision, and whenever possible, a third cannula was inserted into the deep femoral artery. The proximal cannula was connected to the blood line, and oxygenated blood was drawn into the first blood bag where it was mixed with the crystalloid reperfusion solution (blood:reperfusion solution ratio, 6:1). The composition of the crystalloid solution is given in Table I. According to the hemodynamic status of the patient, either 600 mL or 300 mL of blood was taken every cycle.

After the blood-reperfusion solution had been transferred to the second blood bag, the reperfusion line was connected to this second blood bag. After all air had been expelled from the reperfusion line, controlled reperfusion was initiated via the distal cannula. A 12-gauge cannula was inserted into the femoral artery distal of the reperfusion cannula for continuous pressure control. Perfusion pressure was kept strictly <60 mm Hg. In most cases, the blood-reperfusion solution was returned to the leg by gravity alone. If necessary, a pressure-cuffed bag was put around the reperfusion bag to achieve a perfusion pressure close to 60 mm Hg. Perfusion pressure was varied by changing the height of the blood bag.

The procedure was repeated for 30 minutes. The number of cycles performed depended on the flow that could be achieved. After removal of the cannulas, the arteriotomy was closed with direct suture or patch-implantation, and normal blood flow was re-established.



The blood bag perfusion system consists of two blood bags, a crystalloid perfusion solution, and a blood line and perfusion line (two 10-CH cannula are connected to the system).

Table I. Composition of the crystalloid reperfusion solution

NaCl	27.27 mmol/L
THAM (pH 7.5 – 7.7)	54.02 mmol/L
Glutamate × H_2O	34.27 mmol/L
Aspartate × H_2O	34.0 mmol/L
Citric acid × H_2O	2.41 mmol/L
Na citrate × H_2O	13.41 mmol/L
NaH_2PO_4 × H_2O	2.40 mmol/L
Glucose × H_2O	157.96 mmol/L
Allopurinol	7.35 mmol/L

THAM, Tromethamine (tris-hydroxymethyl aminomethane).

Statistical analysis. All numeric data are presented as mean ± SEM. Numeric data were compared using unpaired Student's *t* test, and the χ^2 test was used to compare binomial data. $P \leq 0.05$ was considered statistically significant.

RESULTS

Fifteen patients were included in the study over a 7-month period. Eight were treated with surgical embolectomy alone (group I), and seven had surgical embolectomy and controlled reperfusion (group II). The mean age was 80.5 ± 5.0 years. The site of the arterial occlusion was the iliac artery in eight patients and the common femoral artery in seven patients. There were six women in group I and three women in group II.

In the eight group I patients, five had atrial fibrillation, and four had pre-existing occlusive arterial disease. In the seven group II patients, three had atrial fibrillation, and four had pre-existing occlusive arterial disease. The duration of ischemia was 10.7 ± 1.1 hours in group I patients and 19 ± 2.2 hours in group II patients ($P < .05$).

Preoperative serum CK was 369 ± 107.4 U/L in group I patients and 3257 ± 1888 U/L in group II patients. Serum CK levels 24 hours postoperative were 1630 ± 665 U/L in group I patients and 1778 ± 390 U/L in group II patients. The difference between pre- and postoperative serum CK levels in each group was not statistically significant (Table II).

During the procedure, thrombus could be removed from the occluded vessels in all patients. Back flow of blood from the distal vessels could be achieved as a sign of successful revascularization in all patients. Among the seven patients treated with controlled limb reperfusion, five had cannulation of the superficial femoral artery and the deep femoral artery, and in two patients, only the superficial femoral artery was cannulated. Preparation for controlled reperfusion lasted approximately 5 minutes for cannulation and another 5 minutes for decannulation. This resulted in prolongation of total operating room time of approximately 40 minutes in the patients treated with controlled reperfusion. The volume of crystalloid perfusion solution infused was 520 ± 132 mL.

No intraoperative deaths occurred. Among the patients treated with surgical embolectomy alone, there were three in-hospital deaths caused by acute mesenteric ischemia, myocardial failure, and multiorgan failure. Among the surviving patients, two underwent major amputation, and one patient presented with paralysis of the involved leg at the time of discharge from hospital. No in-hospital deaths occurred among the patients treated with surgical embolectomy and controlled reperfusion, and no major amputation was necessary. One patient had paralysis at the time of discharge from the hospital. Complete functional recovery of the involved leg was achieved in six of the seven patients in group II but in only two of the eight patients in group I ($P < .05$).

DISCUSSION

Acute lower-limb ischemia is probably the most common reason for emergency admission to a vascular surgery unit. The predominant reasons for acute limb ischemia are embolism of cardiac or arterial (eg, aortic aneurysm with adherent thrombus) origin and arterial in situ thrombosis of arteriosclerotic vessels.[1]

Re-establishment of arterial blood flow to the compromised leg is essential for limb salvage. With introduction of the Fogarty catheter in the early 1960s, a simple surgical technique for the revascularization of occluded vessels was introduced into clinical practice. In combination with other surgical techniques for revascularization, such as local thromboendarterectomy and bypass-procedures, vascular surgeons today have a variety of therapeutic options for revascularization of acutely ischemic limbs. Through the last decades, interventional treatment options such as thrombolytic therapy have become another therapeutic option in treating acute limb ischemia.

Despite improvements in revascularization techniques, the results of surgical and interventional treatments have remained unsatisfactory, with high amputation rates and high mortality.[2,19,20] The poor results of revascularization therapy alone may be mainly due to additional reperfusion injury.[6] Reperfusion injury is descriptive of the fact that the reperfusion of ischemic tissue, which is absolutely necessary for tissue salvage, causes further tissue damage that in turn can result in tissue apoptosis and necrosis. However, a prerequisite for evaluating different reperfusion protocols is the achievement of a complete revascularization. Even controlled reperfusion will result in amputation if revascularization cannot be achieved because of obliteration of distal vessels. The therapeutic principle of controlled reperfusion has been used successfully in treating myocardial ischemia and has been shown to improve clinical outcome.[21]

Experimental studies on isolated rat hindlimbs have shown that cellular integrity and biochemical function is preserved after 4 hours of warm ischemia. Severe changes occur after the onset of uncontrolled reperfusion.[22] Reduction of initial reperfusion pressure alone resulted in improved functional and metabolic recovery in an animal model of myocardial ischemia.[23] In an animal model of skeletal muscle ischemia, a reduction of reperfusion blood flow was shown to reduce edema generation and muscle injury.[24]

Controlled reperfusion, with reduced reperfusion pressure and modification of the initial reperfusate, can reduce the local consequences of reperfusion injury such as depletion of high-energy phosphates and local swelling. This is accompanied by improvement in the return of contractile function.[25] The systemic complications of reperfusion, such as release of muscle proteins and potassium into the systemic circulation, could be reduced in an animal model of acute lower-limb ischemia.[12]

By using a simple blood bag reperfusion system, as we did in this study, two main principles of controlled limb reperfusion—a reduction of initial reperfusion pressure and

Table II. Patient data

Pat. No.	*Age (yrs)*	*Site of Occlusion*	*Duration of ischemia (h)*	*CK preop (U/L)*	*CK 24h post-op (U/L)*	*Outcome*
Group I*						
1	63	Iliac artery	16.5	293	209	Normal function
2	87	Iliac artery	13	63	NA	Death (mesenteric ischemia)
3	91	Femoral artery	8	775	2972	Amputation
4	83	Iliac artery	8	NA	NA	Death (myocardial failure)
5	79	Femoral artery	8	460	395	Normal function
6	90	Femoral artery	12	658	2669	Death (multiorgan failure)
7	73	Femoral artery	8	14	15	Amputation
8	79	Femoral artery	12	321	3522	Paralysis
Mean	80.6 ± 3.3		10.7 ± 1.1	369 ± 107	1630 ± 665	
Group II†						
1	80	Iliac artery	8	2262	2420	Normal function
2	89	Iliac artery	12	2218	1015	Normal function
3	92	Iliac artery	18	NA	132	Normal function
4	65	Femoral artery	12	258	2002	Normal function
5	60	Iliac artery	48	12456	3050	Paralysis
6	73	Femoral artery	12	31	161	Normal function
7	72	Iliac artery	23	2321	2017	Normal function
Mean	75.9 ± 4.5		19.0 ± 5.2	3257 ± 1888	1778 ± 390	

CK, Creatine kinase; *NA*, not available.
*Group I (n = 8) had surgical embolectomy alone.
†Group II (n = 7) had surgical embolectomy and controlled reperfusion.

modification of the composition of the initial perfusate—can be achieved in clinical practice with minimal technical effort. As there is evidence that most additional tissue injury caused by uncontrolled reperfusion occurs during the first 20 to 30 minutes, a 30-minute interval for controlled reperfusion was chosen.[26]

Several well-known biochemical changes occur during ischemia and reperfusion. With the onset of ischemia, aerobic metabolism is suspended and anaerobic metabolism is activated. This leads to a breakdown in high-energy phosphates, an increase in intracellular lactate, and intracelluar acidosis.[27] The Krebs cycle loses intermediates.[28] With the start of reperfusion, the washout of lactate and protons from the ischemic tissue leads to an increase in intracellular calcium. An oxidative burst with the generation of oxygen-derived free radicals occurs with the return of oxidative metabolism.[29]

The crystalloid reperfusion solution is aimed to counter these changes. Glucose is added to provide a substrate for anaerobic metabolism and as a hyperosmolar substance to reduce edema generation. Tromethamine is added to buffer ischemic acidosis. With glutamate and aspartate, amino acid precursors of the Krebs cycle are added to ensure more effective oxidative metabolism with the onset of reperfusion. Allopurinol is added to reduce the generation of oxygen-derived free radicals. Sodium citrate is added to reduce intracellular calcium.

The results of this preliminary study show that controlled limb reperfusion using this simplified reperfusion system can be safely performed in any operating room. Controlled limb reperfusion has been used before with good results. All the techniques described so far required the use of a heart-lung machine or roller pumps.[13-15] This required an enormous technical effort and limited the use of controlled reperfusion to large vascular surgery centers. Most patients with acute lower-limb ischemia will not present at centers capable of providing these techniques.

A major difference between the reperfusion system used in this study and the techniques previously described is that our system does not include venous blood drainage of the ischemic limb. After a period of severe ischemia, reperfusion of the involved leg results in the washout of large amounts of muscular waste products into the systemic circulation. This has been described as a part of the reperfusion syndrome and is associated with multiorgan failure and death.[6,30] The rationale for venous blood drainage is to prevent metabolic waste products from entering the systemic circulation. Experimental studies on isolated rat hindlimbs showed that controlled reperfusion significantly reduces the amount of metabolic waste products.[25] On the other hand, drainage of the venous efflux of the ischemic leg requires enormous technical effort and may be associated with acute volume depletion.

The duration of ischemia in the patients included in this study was rather long. The duration of ischemia was significantly longer in the controlled reperfusion group than in the surgical embolectomy alone group, which may be explained by the fact that allocation to either of the two treatment groups was at decision of the responsible surgeon. The surgeons might have believed that the patients with extremely long periods of ischemia might benefit more from controlled reperfusion. No deaths or amputations occurred in the controlled reperfusion group, which contrasts with the results reported in the literature concerning surgical treatment of acute limb ischemia. Clinical studies

on controlled reperfusion for severe lower-limb ischemia have showed good results.[13-17]

To our knowledge there is only one controlled prospective trial that significantly differs from our study, as there were patients with chronic as well as acute lower-limb ischemia enrolled in this study.[16] Some patients in this study were treated with venous drainage of the ischemic leg, which differs from our technique.

CONCLUSION

Our results strongly support the hypothesis that controlled reperfusion can improve outcome after acute severe lower-limb ischemia even though this preliminary study was limited by the small number of patients and the fact that allocation to the treatment groups was not randomized. To prove our preliminary findings in a larger number of patients, we are currently conducting the Controlled Reperfusion of the Acutely Ischemic Limb trial (CRAIL-trial), a prospective, randomized, controlled, multicenter trial.

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